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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/IB97/01130 <b>(22) International Filing Date:</b> 19 September 1997 (19.09.97) <b>(71) Applicants (for all designated States except US):</b> LIGNOCELL HOLZ-BIOTECHNOLOGIE GESELLSCHAFT MBH [AT/AT]; Industriestrasse West 5, A-8605 Kapfenberg (AT). UNIVERSITY OF ABERTAY [GB/GB]; Bell Street, Dundee DD1 1HG (GB). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> MESSNER, Kurt [AT/AT]; Neuwaldeggerstrasse 16/3/7, A-1170 Wien (AT). FLECK, Vinzenz [AT/AT]; Antonienhöhe 36, A-8130 Frohnleiten (AT). BRUCE, Alan [GB/GB]; Siwt, University of Abertay, Bell Street, Dundee DD1 1HG (GB). ROSNER, Bernhard [AT/AT]; Roseggergasse 47/6, A-1160 Wien (AT). <b>(74) Common Representative:</b> MESSNER, Kurt; Neuwaldeggerstrasse 16/3/7, A-1170 Wien (AT).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> PROCESS FOR IMPROVING THE IMPREGNABILITY OF WOOD BY PRETREATMENT WITH FUNGI  <b>(57) Abstract</b>  The object of the invention is a method for improving the impregnability of refractory wood species by pretreatment with fungi of the genera <i>Trichoderma</i> o <i>Gliocladium</i> or with weakly wood-degrading fungi. The pretreatment comprises a thermal treatment of the wood, the addition of a nutrient medium as well as an incubation phase to promote fungal growth. The incubation phase is stopped when the desired depth of penetration of the fungus is reached. The wood is subsequently impregnated with a wood impregnating agent. The typical result of the fungal pretreatment is an increase in the depth of penetration of the impregnating agent to a depth of penetration of 5 mm to 30 mm required depending on the intended use.		

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## PROCESS FOR IMPROVING THE IMPREGNABILITY OF WOOD BY PRETREATMENT WITH FUNGI

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Technical field:

The invention describes a method for improving the impregnability of wood. Various wood species, such as spruce, Douglas fir, fir, oak or acacia, are characterized by favourable properties, such as resistance to checking and splitting as well as low distortion and torsion, straight fibers and minimal branching. These wood species are not widely used because they are difficult to impregnate with chemicals. This is generally true of the heart wood of all wood species. Even if the most efficient impregnating methods are used in wood protection, a wood preservative frequently does not penetrate deeper than 2-3 mm, while the standards in wood protection for timber in European hazard class IV (Ground Contact Timbers) call for a depth of penetration of at least 5-10 mm and of 6 - 30 mm for poles. For example an uptake of at least 63 kg of creosote/m<sup>3</sup> of wood is required for palisades and of 90 kg/m<sup>3</sup> for poles (figure for U.K. is 115Kg/m<sup>3</sup>). This limit cannot be reached with the above-mentioned refractory wood species without additional pretreatment.

At present the method of pretreatment most frequently used in wood protection is the very costly incising method according to which 3 cm deep holes spaced 4 - 8 mm apart are cut into the base of the pole or throughout the sawn timber member in order to promote the penetration of the wood preservative.

The reason for the poor impregnability is the behaviour of the pits during wood drying. Pits are connective openings between the wood cells which permit water transport. The anatomy of the pits is such that the two cell walls on either side of the middle lamella form a dome-

shaped cavity and each has an opening called a pore. At this point the middle lamella is thickened to form the so-called torus. When the wood is dried, this thickened portion  
5 attaches to the border of the pore and seals it irreversibly in refractory wood species. The chemical background of this process has not been ascertained yet.

#### Background Art:

- 10 At present the most common method of improving the impregnability of wood by wood preservatives is the wet storage of wood in ponds for a period of several months, which promotes the development of bacterial populations that open the pits. However, this process cannot be controlled and leads to irregular results.
- 15 A more modern version of this method was developed for the pretreatment of pulpwood in which a mixture of selected strains of bacteria is used and which is said to result in a reduction of blue stain and resin degradation as well as reduction of the cooking time (WO 9636765). A similar effect is achieved in pulping also by pretreating wood chips with the fungus *Ophiostoma piliferum* (Blanchette, Farrell and Burnes 1992, Tappi 75, 102-  
20 106).

In wood protection attempts have been made to restore the pit passage by the action of a pectinolytic enzyme preparation (German Patent DD 292 864 A5). The disadvantage of these processes is that they are designed to degrade only one particular component of  
25 the pits, such as pectin, however apparently several components, not yet determined, are responsible for the pit closure.

Disclosure of the invention:

The subject of the invention is a biological process in which the impregnability of the wood  
5 by wood preservatives is improved by growing fungi on the wood. Preferably fungi of the  
genus

Trichoderma, such as Trichoderma viride SIWT T70, Trichoderma aureoviride SIWT T1 or  
the strains Trichoderma harzianum LC1, LC2, Hypocrea piluliferum LC3, or Gliocladium  
10 roseum LC4 as well as weakly wood-degrading fungi, such as Phanerochaete  
chrysosporium LC5 or Dichomitus squalens LC6 are employed. In the latter case these  
fungi are eradicated after incubation.

The use of living wood-colonizing fungi has the advantage over the use of enzyme  
15 solutions in that enzyme solutions have to be applied by a separate impregnating  
process, which poses a problem in particular with refractory wood species, whereas fungi  
may actively grow into the wood with the help of their filamentous hyphae. Another  
advantage of fungi is that they excrete the necessary mixture of enzymes and other low  
molecular size agents in the vicinity of the pits, which is required for the opening of the pit  
20 passages, but the composition of which has not been determined yet.

The inventive feature of the present process is that for improving the impregnability of  
wood it is inoculated with selected strains of fungi of the genus Trichoderma or  
Gliocladium, or with strains selected from the group of weakly wood-degrading fungi  
25 which are particularly suited to improve the uptake of the impregnating agent by the  
wood. Use of selected strains of Trichoderma or Gliocladium cause no decrease in  
strength of the timber and additionally have the effect of biological wood protection, for  
example Trichoderma harzianum LC1, LC2, Hypocrea piluliferum LC3 (EP 0 615 409 B1).  
The use of weakly wood-degrading fungi only results in limited damage to the wood  
30 during the period of treatment (usually below 10% strength loss).

According to the invention the weakly wood-degrading fungi are eradicated after pretreatment as soon as the desired impregnating aim is achieved to avoid extended  
5 damage to the wood.

The decontaminating and growth promoting measures prior to inoculation enable the applied strains of fungi to rapidly grow into the wood without any appreciable competition by other organisms and to perform their pit opening activity there. The process makes it  
10 possible to use also wood species which are difficult to impregnate without expensive and time consuming mechanical pretreatment processes, such as the incising method, in the same fields of application as easily impregnable wood species thereby significantly expanding the market for such species.

15 Best mode for carrying out the invention:

The method includes a decontamination stage prior to the application of the biological agent to the timber. This stage may not be required however dependant on the timing of the treatment after felling of the timber. A principle of the method according to the  
20 invention is to promote the fungal growth preferably (though not essentially) by thermal decontamination of the wood surface prior to inoculation and by the addition of a growth promoting medium. The decontamination of the wood surface is effected preferably by steam at 100-120°C for 10 seconds - 30 minutes, depending on the degree of contamination, followed by a cooling phase. The growth promoting medium may be  
25 applied separately or jointly with the fungal inoculum. It contains preferably a diluted suspension of cornsteep liquor.

According to the invention inoculation is effected in the case of sporulating fungi, such as the *Trichoderma* and *Gliocladium* species, by applying a spore suspension, and in the case of non-sporulating fungi, such as basidiomycetes, by applying a mycelium suspension to the wood surface. The inoculating suspension may be applied by spraying, dipping, brushing, etc., the inoculum concentration should exceed 100 colony-forming units / ml.

- 10 The incubating phase ideally takes place at the optimum temperature of the respective fungus employed and ranges from 10 to 50°C, but preferably from 20 to 30°C. It may be stopped as soon as the desired depth of penetration of the fungus and the resulting opening of the pits is reached. This objective is reached - depending on the strain of fungus - after a few days to 4 weeks at the most. The resulting loss of wood strength ranges from 0% for *Trichoderma* or *Gliocladium* species to no more than 10% for weakly degrading basidiomycetes depending on the fungal strain (see results table).

If weakly wood-degrading fungi are used, the fungus grown into the wood is eradicated according to the invention at the end of incubation. This may be done, depending on the type of method used, either in the course of wood drying in drying kilns at elevated temperatures of 40-100°C or by steaming of the wood at 100-120°C or by a fungicide applied after fungal colonisation. Furthermore, the fungus may be eradicated in the course of pressure treatment in which temperatures of up to 160°C are reached.

- 25 The subsequent impregnating process may be a pressure treatment (high pressure or low pressure process), non-pressure treatment (dip treatment), or special process. The impregnating agents to be employed are not limited to wood preservatives, but comprise all chemical substances designed for impregnation, such as fire protectants, synthetic resins, and chemicals designed for wood modification etc.

Example:

Spruce logs stripped of the bark (*Picea abies*, 1 m length, 100 to 200% wood moisture content) were subjected to a decontamination treatment by steaming for 10 minutes and  
5 were subsequently cooled down to ambient temperature.

The inoculum fungi *Trichoderma viride* SIWT T70, *Trichoderma aureoviride* SIWT T1, *Trichoderma harzianum* LC1, *Trichoderma harzianum* LC2, *Trichoderma harzianum* LC3, *Gliocladium roseum* LC4, *Phanerochaete chrysosporium* LC5 *Dichomitus squalens* LC6,  
10 were grown in Petri dishes on 2% malt agar and blended in a sterile mixer in a 2% solution of cornsteep liquor. The concentrated fungal suspensions were diluted in a growth promoting medium containing cornsteep liquor and were used as an inoculum. Inoculation was effected by dipping 10 spruce logs into the microbial inoculum of each test fungus in the respective mycelium suspension. The strains were subsequently  
15 incubated in rooms with constant climate at the optimum temperatures for each test fungus for 1, 2, 3 or 4 weeks, respectively.

After the incubation period the spruce logs were dried in a drying kiln at temperatures between 50 and 65°C for a period of 12 days to 10 - 20 % wood moisture content. Ten  
20 logs not inoculated with fungi were dried under the same conditions to serve as controls.

The pretreated logs and the controls were impregnated with creosote in the same way according to an industrial pressure treatment process designed for pine wood. They were subsequently cut apart and the depth of penetration measured. Additionally test wood  
25 blocks were cut out and subjected to strength tests (ISO Standard No.3349) as well as analyzed for the loading of the wood preservative.



## Results:

		weeks of incubation	depth of penetration (cm)	creosote loading kg/m <sup>3</sup>	strength loss (%)
5	SIWT T70	3	2,7 - 3,0	150	0
10	SIWT T1	3	2,0 - 2,7	160	0
	T. harz. LC1	3	2,5 - 2,9	160	0
	T. harz. LC2	3	2,0 - 2,7	155	0
15	H. pil. LC3	3	2,6 - 3,0	160	0
	Gl. ros. LC4	3	2,1 - 2,9	160	0
20	Ph. chr. LC5	1	2,3 - 3,0	240	0
		2	2,3 - 3,2	260	10
		3	2,5 - 3,2	260	10
	D. squ. LC6	1	1,0 - 1,5	130	0
25		2	2,3 - 2,8	150	10 -15
		3	2,3 - 3,0	250	10 -15
	Controls		0,15 - 0,95	*)	0

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\*) cannot be tested because penetration is not deep enough.

A depth of penetration of 2 - 3 cm correlates with a total penetration of the sap wood area of the logs with creosote.

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### Industrial Applicability:

The method according to the invention is applicable in all those areas in which the capability of certain wood species to take up any impregnating agent does not guarantee a particular quantity of uptake or depth of penetration. The main field of application is wood protection, as e.g. when using spruce wood (*Picea* spp.) for poles or palisades. Without any prior opening of the pit passages and clearing of blockage materials in the

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ray parenchyma tissues the uptake of the wood preservatives required for protection of timber in soil contact situations can only be reached by very costly conventional physical processes. Preservative treatment levels required to meet national standards can however be achieved by the pretreatment according to the invention. Similarly, sawn construction timbers can be more properly treated with preservatives using the method.

However, the invention also extends to the impregnation of wood with other impregnating agents, such as synthetic resins, or to agents for the chemical modification of wood.

Applicant's or agent's file reference number	International application No. <b>PCT/IB 97 201130</b>
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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>3/7/11</u> , line <u>8, 9, 10, 11, 28/8-26/10, 13, 14, 17, 20, 26</u>	
B. IDENTIFICATION OF DEPOSIT	
Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution Centraalbureau voor Schimmelcultures	
Address of depositary institution (including postal code and country) CBS P.O.Box 273 NL 3740 AG BAARN	
Date of deposit 1) SEPT. 4, 1997 2) SEPT. 4, 1997 3) SEPT. 17, 1997 4) SEPT. 17, 1997 5) SEPT. 16, 1997	Accession Number 1) CBS 100068 2) CBS 100069 3) CBS 100071 2) CBS 100072 3) CBS 100073
C. ADDITIONAL INDICATIONS (leave blank if not applicable)	
This information is continued on an additional sheet <input type="checkbox"/>	
1) TRICHODERMA AUREOVIRIDE SIWT T7 2) TRICHODERMA VIRIDE SIWT T70 3) GLIOCLADIUM ROSEUM LC4 4) PHANEROCHAETE CHRYSOSPORIUM LC5 5) DICHOMITUS SQUALENS LCB	
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A. The indications made below relate to the microorganism referred to in the description on page <u>3/7/1.1</u> , line <u>8, 9, 10, 11, 28/3-26/10, 13, 14, 17, 20, 26</u>	
B. IDENTIFICATION OF DEPOSIT	
Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution Centraalbureau voor Schimmelcultures	
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Date of deposit <u>1), 2), 3) Nov. 4, 1991</u>	Accession Number <u>1) CBS 595.91</u> <u>2) CBS 596.91 3) 597.91</u>
C. ADDITIONAL INDICATIONS (leave blank if not applicable)	
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1) TRICHODERMA HARZIANUM LC 1 2) TRICHODERMA HARZIANUM LC 2 3) HYPOCREA PILULIFERA LC 3	
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Claims:

1. A method for improving the impregnability of wood, characterized in that the wood to  
5 be impregnated with any chemical is made accessible by fungal inoculation comprising  
colonizing it with a fungus or a mixture of fungi or a mixture of fungi and other  
microorganisms.
2. The method of claim 1, characterized in that the fungi used are selected from the  
10 genus *Trichoderma* or its teleomorphic states like *Hypocrea*.
3. The method according to claims 1 and 2, characterized in that the fungi used are the  
strains *Trichoderma viride* SIWT T70, *Trichoderma aureoviride* SIWT T1, *Trichoderma*  
*harzianum* LC 1, *Trichoderma harzianum* LC 2, *Hypocrea piluliferum* LC 3.
- 15 4. The method according to claim 1, characterized in that the fungi used are selected  
from the genus *Gliocladium*.
5. The method according to claims 1 and 4, characterized in that the fungus used is the  
20 strain *Gliocladium roseum* LC4.
6. The method according to claim 1, characterized in that the fungi used are weakly  
wood-degrading Basidiomycetes, Ascomycetes or Deuteromycetes.
- 25 7. The method according to claims 1 and 6, characterized in that the fungus used are the  
strains *Phanerochaete chrysosporium* LC5 and *Dichomitus squalens* LC6.

8. The method according to one of claims 1 to 7, characterized in that the wood is  
5 subjected to a chemical or thermal pretreatment for decontamination prior to fungal  
inoculation, preferably with steam at a temperature of from 100-120°C, and is  
subsequently cooled.
9. The method according to one of claims 1 to 8, characterized in that the wood to be  
10 colonized by the fungus is supplied with a growth promoting medium, preferably  
containing cornsteep liquor.
10. The method according to one of claims 1 to 9, characterized in that after fungal  
inoculation an incubation phase adapted to the fungi used takes place, the incubating  
15 temperature being from 10 to 50°C, preferably from 20 to 30°C.
11. The method according to one of claims 6 to 10, characterized in that the fungus is  
eradicated after colonizing the wood, preferably by heat.
- 20 12. Any wooden member treated according to the method according to one of claims.

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/IB 97/01130

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 B27K3/00 B27K5/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 B27K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FR 2 705 607 A (DEMTOS SA TONNELLERIE) 2 December 1994 see page 40, line 20-28 ---	1-12
A	EP 0 001 540 A (ENFORS SVEN OLOF ; MOLIN NILS (SE); MONTELIN EUGEN (SE)) 18 April 1979 see page 4, line 18-25; claim 1 ---	1
A	WO 93 08694 A (LIGNOCELL HOLZ BIOTECH) 13 May 1993 cited in the application see the whole document ---	1-12
A	GB 2 239 800 A (DUNDEE INST OF TECHNOLOGY ; QUARMBY ARTHUR (GB)) 17 July 1991 see claims ---	1-3, 12
	-/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/IB 97/01130

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 3 486 969 A (NILSSON THOMAS ET AL) 30 December 1969 see claims ----	1,4,12
A	DD 292 864 A (WEB WISS TC.HOLZ) 18 April 1991 cited in the application -----	



# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/IB 97/01130

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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